Activated T lymphocytes induce degranulation and cytokine production by human mast cells following cell-to-cell contact

Siba P. Bhattacharyya,* Ilana Drucker,† Tamar Reshef,† Arnold S. Kirshenbaum,* Dean D.Metcalfe,* and Yoseph A. Mekori†

The Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; and †Allergy-Clinical Immunology Unit, Department of Medicine, Meir General Hospital, Sackler School of Medicine, Tel Aviv University, Israel

Abstract: Activated mast cells reside in close apposition to T cells in some inflammatory processes. In this study, we analyzed whether this close physical proximity affects human mast cell degranulation and cytokine release. Thus HMC-1 human mast cells or primary bone marrow-derived human mast cells were cocultured with activated and with resting T cells. Mast cells cocultured with activated T cells released histamine and β-hexosaminidase and produced tumor necrosis factor α (TNF-α), an effect that peaked at 20 h. Kinetics of histamine release paralleled the formation of heterotypic aggregates. Separation of the two cell populations with a porous membrane prevented mediator release and TNF-α production. Addition of the PI3-kinase inhibitor, wortmannin, inhibited the heterotypic adhesion-associated degranulation but not TNF-α production. These data thus indicate a novel pathway through which human mast cells are activated to both release granule-associated mediators and to produce cytokines in association with heterotypic adhesion to activated human T cells. *J. Leukoc. Biol.* 63: 337–341; 1998.

Key Words: *heterotypic adhesion · tumor necrosis factor α · histamine*

INTRODUCTION

Mast cells are known to be essential resident effector cells in the elicitation of the allergic response. IgE-sensitized mast cells, upon encounter with specific antigen that is recognized by their receptor (FcεRI)-bound IgE, secrete bioactive mediators that facilitate the development of allergic inflammation [1]. Morphological studies have documented that mast cells also undergo degranulation during T cell-mediated inflammatory processes as observed in helminth infections, cutaneous delayed hypersensitivity, graft-versus-host reactions, hypersensitivity pneumonitis, sarcoidosis, and rheumatoid arthritis [reviewed in refs. 2 and 3]. Furthermore, morphological studies have also revealed that mast cells reside in close physical proximity to T cells in inflamed allergic tissues and at sites of parasitic infections [4, 5]. This close apposition between mast cells and T cells have led investigators to propose a functional relationship between these two cell populations that might facilitate the elicitation of the immune response [6].

T cell-mast cell interactions have been shown to be bidirectional, fulfilling mutually regulatory and/or modulatory roles, including influences on cellular processes such as growth, proliferation/activation, migration, and Ag presentation [7–9]. So far, the inductive effect of T cells on mast cell activation and degranulation has been attributed to the biological effects of certain cytokines released from the former. Specific cytokines have been shown to affect mast cell degranulation either directly (i.e., macrophage inflammatory protein-1α) [10] or indirectly by priming mast cells and potentiating the effect of classical secretagogues such as IgE plus Ag or complement components [11]. We have recently reported that murine mast cells are in addition stimulated to degranulate in association with direct contact with activated murine T cells [12]. In this study we demonstrate that human mast cells in contact with activated human T cells are induced to both release granule-associated histamine or β-hexosaminidase, and produce TNF-α, describing a heretofore unrecognized mast cell activation pathway through which human mast cells may be recruited in T cell-mediated inflammatory processes.

RESULTS

To initially explore the question of whether human lymphocytes are able to induce mast cell activation/degranulation through a mechanism requiring cell-to-cell contact, we first analyzed the effect of T cell activation on granule-associated mediator release by mast cells. Thus, Jurkat T cells were pre-incubated for 30 min in the presence of phorbol myristate acetate (PMA; 50 ng/mL), washed (three times), and cocultured with an equal number (1×10^5/well) of HMC-1 mast cells [13] for 16–20 h. At the end of incubation, degranulation was measured by either granule-associated β-hexosaminidase or histamine release (enzyme immunoassay kit; Immunotech, Marseille, France). As

Abbreviations: TNF-α, tumor necrosis factor α; PMA, phorbol myristate acetate; ECM, extracellular matrix.

Correspondence: Dean D. Metcalfe, M.D., NIH/NIAID/LAD, Building 10, Room 11C205, 10 Center Drive MSC 1881, Bethesda, MD 20892-1881.

Received September 24, 1997; revised December 26, 1997; accepted December 29, 1997.

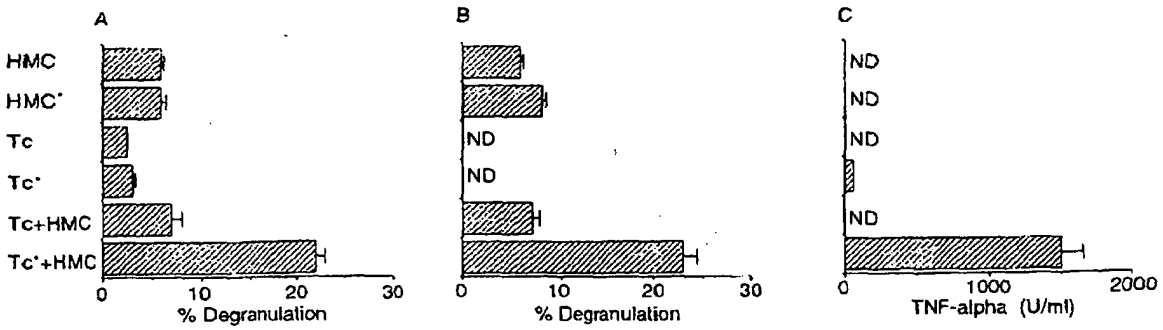

Fig. 1. Effect of PMA activated T cells (Tc) on β-hexosaminidase release and TNF-α production by HMC-1 mast cells. Jurkat T cells (A) or freshly isolated human peripheral blood T cells (B, C) were incubated in the presence (*) or absence of PMA at 50 ng/mL for 30 min, washed (three times), and added to wells containing mast cells. Mediators in the supernatants of the cocultures were measured at 16–20 h of incubation. β-Hexosaminidase (A, B) was measured spectrophotometrically by assaying the cleavage of its substrate *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide. Degranulation is expressed as percentage of the cells' total mediator content obtained by lysis of cells with Triton X-100. TNF-α (C) was measured by a bioassay as described in the text. Data presented as mean ± SEM of six independent experiments performed in duplicate. ND, not detected.

shown in **Figure 1A**, non-activated T cells induced only a minimal increase in degranulation from mast cells; however, PMA-activated Jurkat cells induced significant mast cell degranulation as measured by β-hexosaminidase release from HMC-1 mast cells (22 ± 1 compared with 7 ± 1% degranulation of HMC-1 co-cultured with resting T cells; $P < 0.001$). When HMC-1 mast cells were treated with PMA alone for 30 min, i.e., without the presence of T cells, no enhancement of degranulation was detected (Fig. 1A). As with the Jurkat T cells, activated (PMA treated), but not resting, freshly isolated peripheral blood T cells (IsoCell human T cell isolation kit; Pierce, Rockford, IL) induced β-hexosaminidase release from HMC-1 mast cells (Fig. 1B). Freshly isolated PMA-stimulated T cells were somewhat more effective than stimulated Jurkat T cells in releasing β-hexosaminidase if lymphocyte-derived β-hexosaminidase is taken into account. We next examined co-cultures for evidence of cytokine production. We found that activated T cell-induced mast cell degranulation was also associated with a significant TNF-α production as measured by cytotoxic activity against L929 cells, with the use of an MTT assay as described previously [14] (Fig. 1C). Because T cells were isolated from multiple donors, and because the HMC-1 mast cells lack FcεRI [13], the data strongly suggests that activated T cells induce mast cell degranulation and TNF-α production through activation pathways that are MHC independent and do not involve interaction with FcεRI.

Activated mast cells are known to form heterotypic aggregates with T lymphocytes. We therefore examined whether contact between T cells and mast cells is required for the induction of activated T cell-dependent mast cell mediator release. For this purpose we first analyzed the kinetics of mediator release induced by PMA-activated T cells. Thus, HMC-1 cells were cocultured with PMA-pretreated Jurkat cells for an incubation period that lasted 24 h. Sample supernatants were collected at several time points for the measurement of β-hexosaminidase release. As shown in **Figure 2A**, β-hexosaminidase was first detected at 4 h, with a maximal release at 20–24 h. Once again, enhanced release was detected

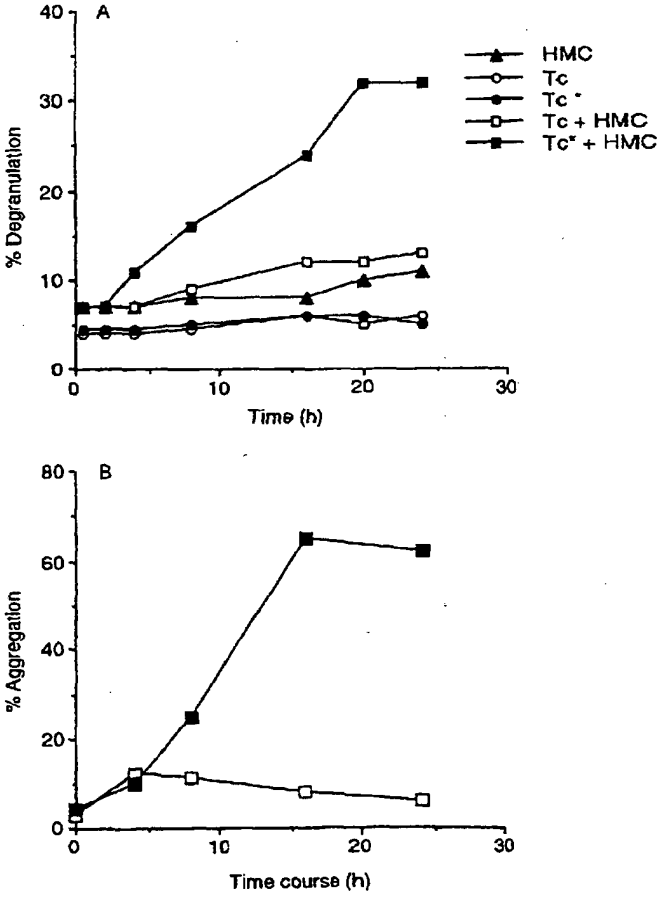

Fig. 2. Kinetics of β-hexosaminidase release and heterotypic aggregate formation induced by activated T cells. PMA-treated Jurkat T cells (*) were co-cultured with HMC-1 mast cells. (A) Supernatants were collected at various time points and degranulation calculated as above. Each value represents the mean of two independent experiments. (Variation between the two experiments was <5%). (B) Percentage of heterotypic aggregate formation was calculated as % aggregation = (1 − number of free cells/number of total cells) × 100 [15].

only in co-cultures that included activated Jurkat T cells. Cultures in which HMC-1 mast cells were incubated alone or with non-activated T cells did not show increased β-hexosaminidase release. Kinetics of β-hexosaminidase release paralleled the formation of heterotypic aggregates as measured by phase-contrast microscopy using a calibrated ocular grid as previously described [15] (Fig. 2B).

Two different experimental approaches were employed to further elucidate the role of intercellular contact between T cells and mast cells in the induction of degranulation. In this set of experiments, Jurkat cells or freshly isolated peripheral blood T cells were activated with immobilized, plastic-bound anti-CD3 mAb (96-well plates precoated overnight at 4°C with 25 μg/mL of the Ab or with isotype control). As can be seen in **Figure 3A**, Jurkat T cells that were preincubated with the immobilized IgG isotype control did not stimulate significant histamine release when compared with background release by resting mast cells (MC alone; Fig. 3A). Activation of T cells with anti-CD3, however, induced a significant increase of histamine release from the co-cultured mast cells (30 ± 2%) compared with either resting nontreated mast cells or with mast cells cocultured with nonstimulated T cells (10 and 10.2% histamine release, respectively). We have also reproduced these results using primary human bone marrow-derived mast cells [16]. These cells, when cocultured with immobilized anti-CD3-activated T cells, demonstrated 28.0% β-hexosaminidase release compared with 2.8% for resting mast cells or a 3.0% release of mast cells cocultured with immobilized IgG-treated T cells. As shown in Figure 3B, anti-CD3-activated freshly isolated T cells did not induce β-hexosaminidase release from HMC-1 mast cells if these two cell populations were separated by a microporous membrane (Transwell cell culture chamber, Costar, Cambridge, MA; pore size 0.4 μm). Separation of the two cell populations also prevented TNF-α production (not shown). When supernatants from either resting or activated Jurkat T cells, pretreated with immobilized IgG or anti-CD3 mAb, respectively, were added to HMC-1 cells at 50% (vol/vol), no histamine release (above background release of resting mast cells) could be detected at 20 h of incubation (not shown). These results were consistent with the conclusion that cell-to-cell contact was essential to promote histamine release induced by activated T cells cocultured with mast cells.

To initially investigate the signal transduction pathway involved in this heterotypic adhesion-induced mast cell activation, we pretreated HMC-1 mast cells with the phosphatidylinositol 3-kinase (PI3-kinase) inhibitor wortmannin (100 nM for 10 min, followed by three washes) before co-culturing with the T cells. As shown in **Figure 4**, pretreatment with wortmannin did not affect baseline β-hexosaminidase release from resting cells, however, it virtually blocked the release from mast cells cocultured with PMA-activated T cells. In contrast, there was no noticeable effect of wortmannin on TNF-α production by mast cells (Fig. 4). These results are compatible with recent reports demonstrating that, unlike histamine release, cytokine production in mast cells does not involve the PI3 activation pathway [17].

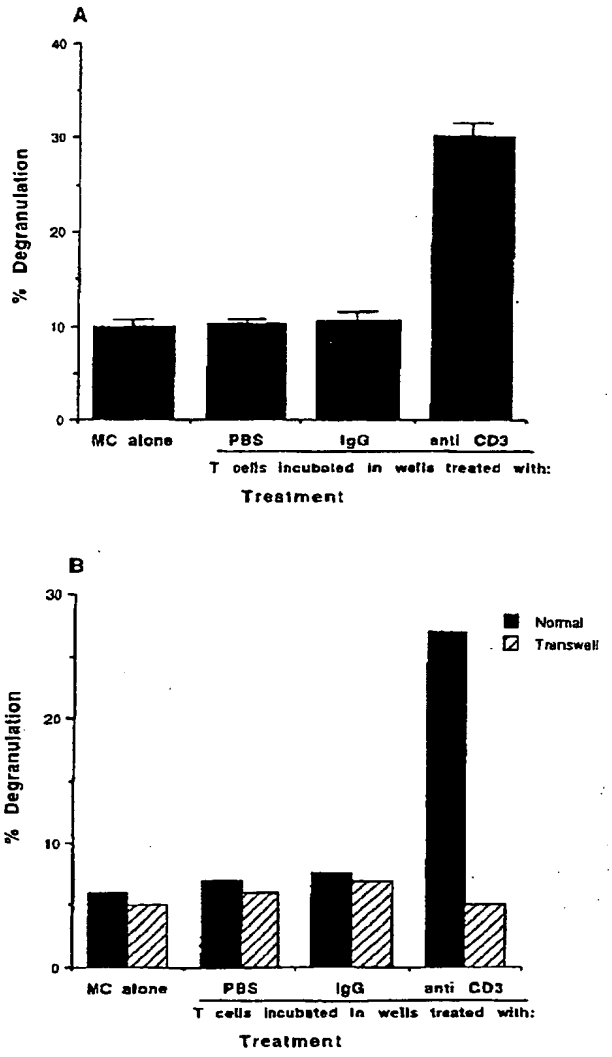

Fig. 3. T cells activated with anti-CD3 mAb induce histamine release from HMC-1 mast cells; effect of semipermeable barrier. (A) Jurkat T cells were added in wells coated with or without IgG or anti-CD3 mAb and incubated with mast cells for 18 h. Supernatants were collected and histamine content measured using an enzyme-linked immunosorbent assay. Each value represents the mean ± SEM of three independent experiments, each performed in triplicate. Immobilized anti-CD3 Ab did not affect mast cells (not shown). (B) Freshly isolated T cells were incubated in wells pre-coated with anti-CD3 mAb or with the isotype IgG control. HMC-1 cells were added to these wells and co-cultured with the T cells (normal) or added in wells separated into two compartments using the Transwell cell culture chamber. Supernatants were collected at 18 h and degranulation assessed by measuring β-hexosaminidase. Data are the means of two independent experiments.

DISCUSSION

The demonstration that mast cells express multiple adhesion molecules [13, 18] has provided insight into possible adhesive interactions between mast cells and extracellular matrix (ECM) components or other cell types. In addition to facilitating cell migration, adhesion of mast cells to ECM components transduces a variety of intracellular signals that regulate cell

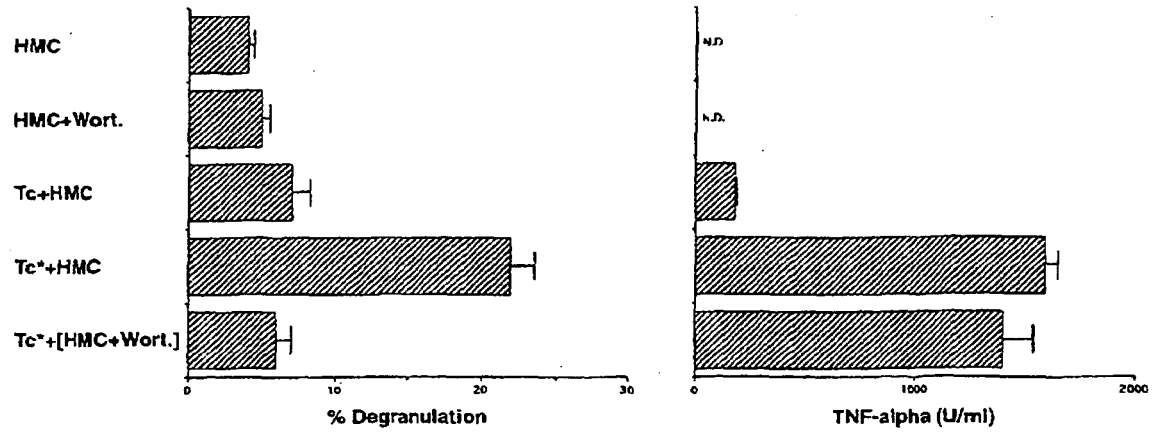

Fig. 4. *Effect of wortmannin on activated T cell-induced mast cell degranulation.* HMC-1 mast cells were pretreated with wortmannin (100 nM for 10 min, followed by three washes) and then added to PMA-activated or nonactivated freshly isolated T cells. β-Hexosaminidase (left) and TNF-α production (right) were measured in the supernatants at the end of 8 h incubation. Data presented as mean ± SEM of two independent experiments performed in triplicate.

function. These signals include protein tyrosine phosphorylation, phosphoinositide hydrolysis, changes in intracellular calcium, and up-regulation of the expression of several genes [reviewed in ref. 19]. Thus, the IL-3-induced DNA synthesis and proliferation of murine bone marrow-derived mast cells is augmented by integrin-mediated adherence to vitronectin [20]. Mast cell exocytosis is enhanced by adhesion to ECM or on interaction with fibroblasts [19]. The mechanism by which cell adhesion regulates secretion is not fully understood; however, cell attachment results in cytoskeletal changes and changes in protein tyrosine phosphorylation, all of which might directly influence degranulation [19]. This study suggests that adhesion of mast cells to activated T lymphocytes similarly induces mast cell degranulation and cytokine production. Other heterotypic adhesion-induced effects on mast cell activation have recently been reported. Thus, intercellular contact between mast cells and activated T cells has been found to induce promoter activity of the TCA3 gene in mast cells [21]. Similarly, it has been reported that incubation of HMC-1 cells with membranes purified from anti-CD3 activated T cells results in the induction of interleukin 8 transcripts but not for other proinflammatory cytokines, including TNF-α [22]. The latter observation suggests that activation of mast cells aggregated to intact activated T cells is not reproduced using cell membranes.

Adhesion-dependent activation has been shown in other cell systems. The adhesion pathway mediated by LFA-1 and its ligand ICAM-1 is one of the best-studied costimulatory pathways in T cells [23]. It has been well demonstrated that LFA-1-dependent adhesion of T cells to ICAM-1 requires activation of protein kinase C by triggers such as phorbol esters or by cross-linking cell surface molecules such as CD3 [24]. It has been shown that costimulation provided for anti-CD3-mediated proliferation of T cells involves an extended LFA-1/ICAM-1 interaction leading to signal transduction events that result in prolonged (>4 h) inositol phospholipid hydrolysis and a sustained increase in free cytosolic calcium level [25]. This observation may be relevant to the relatively late onset of the effects of T cell contact on mast cell activation and mediator release observed in our study. It is also possible that adhesion-induced mast cell activation by T cells involves induction of new proteins or other mediators that, in turn, generates a new series of signals leading to mast cell activation and mediator release. Taken together, the morphological studies showing mast cells in close apposition to T cells, and the data presented in this study, indicate a heretofore unappreciated pathway through which mast cells can be activated to release granule-associated mediators and produce cytokines when involved in T cell-mediated inflammation.

REFERENCES

1. Galli, S. J. (1997) Complexity and redundancy in the pathogenesis of asthma: reassessing the role of mast cells and T cells. *J. Exp. Med.* **186**, 343–347.
2. Mekori, Y. A., Zeidan, Z. (1990) Mast cells in nonallergic immune responses in vivo. *Isr. J. Med. Sci.* **26**, 337–341.
3. Nechushtan, H., Razin, E. (1996) Regulation of mast cell growth and proliferation. *Crit. Rev. Oncol. Hematol.* **23**, 131–150.
4. Friedman, M. M., Kaliner, M. (1985) In situ degranulation of human nasal mucosal mast cells: ultrastructural features and cell-to-cell association. *J. Allergy Clin. Immunol.* **76**, 70–82.
5. Smith, T. J., Weiss, J. H. (1996) Mucosal T cells and mast cells share common adhesion receptors. *Immunol. Today* **17**, 60–63.
6. Mecher, S., David, B. (1997) Unravelling the mast cell dilemma: culprit or victim of its generosity? *Immunol. Today* **18**, 212–215.
7. Razin, E., Ihle, J. N., Seldin, D., et al. (1984) Interleukin 3: a differentiation and growth factor for the mouse mast cells that contains chondroitin sulfate E. *J. Immunol.* **132**, 1479–1486.
8. Hershkoviz, R., Lider, O., Baram, D., Reshef, T., Miron, S., Mekori, Y. A. (1994) Inhibition of T cell adhesion to extracellular matrix components by histamine: a role for mast cell degranulation products. *J. Leukoc. Biol.* **56**, 495–501.
9. Fox, C. C., Jewell, S. D., Whitacre, C. C. (1994) Rat peritoneal mast cells present antigen to a PPD-specific T cell line. *Cell. Immunol.* **153**, 253–258.
10. Alam, R., Forsythe, P. A., Stafford, S., Lett-Brown, M. A., Grant, J. A. (1992) Macrophage inflammatory protein-1α activates basophils and mast cells. *J. Exp. Med.* **176**, 781–786.
11. Levi-Schaffer, F., Segal, V., Shalit, M. (1991) Effect of interleukins on connective tissue type mast cells co-cultured with fibroblasts. *Immunol.* **72**, 174–180.
12. Mekori, Y. A., Inamura, N., Metcalfe, D. D. (1996) Induction of mast cell degranulation by heterotypic aggregation with T cells. *J. Allergy Clin. Immunol.* **97**, 263A.

13. Nilsson, G., Blom, T., Kusche, M., Kjellen, L., Butterfield, J. H., Sundstrom, C., Nilsson, K., Hellman, L. (1994) Phenotypic characterization of the human mast cell line HMC-1. *Scand. J. Immunol.* **39**, 489–498.
14. Gordon, J. R., Galli, S. J. (1990) Mast cells as a source of both preformed and immunologically inducible TNF-α. *Nature* **346**, 274–276.
15. Dang, L. H., Rock, K. L. (1991) Stimulation of B lymphocytes through surface Ig receptors induces LFA-1 and ICAM-1-dependent adhesion. *J. Immunol.* **146**, 3273–3278.
16. Kirshenbaum, A. S., Goff, J. P., Kessler, S. W., Mican, J., Zsebo, K. M., Metcalfe, D. D. (1992) The effect of IL-3 and stem cell factor on the appearance of mast cells and basophils from CD34$^+$ pluripotent progenitor cells. *J. Immunol.* **148**, 772–777.
17. Marquardt, D. L., Alongi, J. L., Walker, L. L. (1996) The phosphatidylinositol 3-kinase inhibitor wortmannin blocks mast cell exocytosis but not IL-6 production. *J. Immunol.* **156**, 1942–1945.
18. Guo, C. B., Kugey-Sobotka, A., Lichtenstein, L. M., Bochner, B. S. (1992) Immunophenotyping and functional analysis of purified human uterine mast cells. *Blood* **79**, 708–712.
19. Hamawy, M. M., Mergenhagen, S. E., Siraganian, R. P. (1994) Adhesion molecules as regulators of mast cell and basophil function. *Immunol. Today* **15**, 62–66.
20. Bianchine, P. J., Burd, P. R., Metcalfe, D. D. (1992) IL-3-dependent mast cells attach to plate-bound vitronectin. Demonstration of augmented proliferation in response to signals transduce via cell surface vitronectin receptors. *J. Immunol.* **149**, 3665–3671.
21. Oh, C. K., Metcalfe, D. D. (1996) Activated lymphocytes induce promoter activity of TCA-3 gene in mast cells following cell-to-cell contact. *Biochem. Biophys. Res. Commun.* **221**, 510–514.
22. Kirshnaswamy, G., Lakshman, T., Miller, A. R., Srikanth, S., Hall, K., Huang, S-K., Suttles, J., Smith, J. K., Stout, R. (1997) Multifunctional cytokine expression by human mast cells: regulation by T cell membrane contact and glucocorticoids. *J. Interferon Cytokine Res.* **17**, 167–176.
23. Hogg, N., Harvey, J., Cabnas, C., Landis, C. (1993) Control of leukocyte integrin activation. *Am. Rev. Respir. Dis.* **148**, S55–S59.
24. Dustin, M. L., Springer, T. A. (1989) T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* **341**, 619–622.
25. Van Seventer, G. A., Bonvini, E., Yamada, H., Conti, A., Stringfellow, S., June, C. H., Shaw, S. (1992) Costimulation of T cell receptor/CD3 mediated activation of resting human CD4$^+$ T cells by LFA-1 ligand ICAM-1 involves prolonged inositol phospholipid hydrolysis and sustained increase of intracellular Ca^{2+} levels. *J. Immunol.* **149**, 3872–3880.